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Phytosanitary rules for grapevine (*Vitis vinifera* L.) propagation material introduction into EU for germplasm conservation and scientific purposes

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Foreword

The main purpose of the present proposal is to simplify and make more consistent the regulation for grapevine (*Vitis vinifera* L.) propagation material introduction into EU for germplasm conservation, scientific purposes and breeding.

Germplasm collections should be considered with a particular attention by the phytosanitary regulations. They represent a precious source of genetic resources. Not rarely a cultivar is maintained just in one collection.

A sustainable strategy for germplasm conservation, evaluation and utilization should include firstly a wider application of diagnostic tools and disease control in the germplasm repositories. Moreover the germplasm mobilization does represent a real opportunity to reduce the risk of losing biodiversity. In this context the present specific protocol intends to regulate the germplasm circulation among institutions holding germplasm collections, including specific quarantine procedure management.

The present protocol is based on the FAO/IBPGR Technical Guidelines for the Safe Movement of Grapevine Germplasm (1991) taking however into account that the guideline does not include the most recent knowledge and methods currently available and used. Moreover it should be noted that:

- the Technical guidelines are very complicated, not applicable for practical and fast plant material introduction into EU because the evaluation and therapy of infected material are rather difficult, time consuming and expensive; moreover the effect of the chemotherapy may be critical on the phenotypic trueness to type and stability;
- more than one pesticide recommended by the Technical guidelines is not yet allowed;
- the therapy procedures should be used just in case of a very unique material.

Reference regulation of plant introduction into EU

The present protocol acknowledges that:

- transfer of plant material into EU is regulated by Directive 2000/29/EC;
- introduction of the grapevine plant material into EU is prohibited with exception of the material transfer for scientific purposes and breeding;
- the conditions under which plants, and plant products may be introduced into or moved within the Commu-

nity for trial or scientific purposes and for work on varietal selections is regulated by Commission Directive 2008/61/EC of 17 June 2008.

Protocol for safe introduction into EU of grapevine propagation material for germplasm conservation, scientific purposes and breeding

The present protocol refers only to dormant woody cuttings. The plant material transferred into EU should be free from EU quarantine organisms. Mother plants evaluation in the country of origin have to be based on:

- visual assessment of disease symptoms;
- ELISA specific tests;
- PCR-based diagnostic tools – EU approved and/or available methods for pathogen detection and identification.

Based on the negative test results in detection of quarantine organisms, the plant material will be subjected to the procedure for the introduction of plant material into the EU, in accordance with in force regulations.

Procedure of the plant material introduction into EU

Prerequisites before application for material introduction approval: Approved quarantine conditions in relation to qualifications of the personnel, quarantine containment conditions of the location and facilities.

Progressive steps of the approval of plant material introduction – duties for National Plant Protection Organization of Importer Member State (IMS) or Exporter Third Country (ETC):

1. Application for introduction of plant material (IMS).
2. Approval of the planned activities concerned (IMS). The nature and objectives of the activities for which the material is to be introduced or moved shall have been examined by the responsible official body of importing Country and found to comply with the concept of trial or scientific purposes and or work on varietal selections provided for under Directive 2008/61/EC.
3. Approval of post-entry quarantine station (IMS). Quarantine site and personnel should be approved by official body.
4. Field and laboratory controls on propagating materials and related mother plants (ETC).

5. Approval letter of authority for introduction of plants for trial or scientific purposes and for work on varietal selections (IMS). The responsible official body shall limit the quantity of material to an amount that is adequate for the approved activities and in any case the amount shall not exceed quantities which have been determined having regard to available quarantine containment facilities. The import permit shall be released on the basis of the approval of the activities, and on the assurance that quarantine containment conditions shall be applied during movement and detection of the material.
6. Export Phytosanitary Certificate (PC) issue (ETC). The PC shall indicate an additional declaration stating that “the consignment complies with Directive 2008/61/EC”.
7. Phytosanitary inspection at the Border Inspection Point and introduction in UE, to post-entry quarantine approved station (IMS).
13. Production of rooted plants, if needed .
14. Field planting in guarded conditions including evaluation of vector’s presence.
15. Monitoring during experimental or conservation activity.

D. Additional remarks :

It has to be underlined that the release of any imported propagating material for circulation and trade purposes should provide further and closer investigation about sanitary status of the materials that have to include:

16. Deep sequencing (Next Generation Sequencing and BLAST) to detect any known harmful organism (HOs);
17. Pest Risk Analysis for EU, per each HO. Only for potentially HOs for EU should be provided adequate restrictions or phytosanitary measures.

Annexes

Specific procedures for dormant cuttings introduction

A. Pre-shipment controls :

1. Field controls on mother plants at the place of origin, in appropriate timing.
2. Selection (exclusion of all symptomatic vines).
3. ELISA/PCR tests for grapevine known harmful pathogens. Analysis results should be indicated in additional statement in phytosanitary certificate.
4. Collecting woody canes.
5. Dipping in appropriate insecticide and fungicide.
6. Delivery of appropriate Phytosanitary Certificate (specific additional declaration) for each consignment.

B. Entry controls :

7. Entry controls performed at approved Border Inspection Points (BIP).

C. Post-entry controls :

8. Arrival at approved “Post Entry Quarantine Station”, under official control by National Plant Protection Organization (NPPO).
9. Further accurate Visual Testing (insects, mites, epiphytic bacteria and fungi);
10. Detecting, isolation and identification of epiphytic bacteria and fungi (e.g. Biolog).
11. ELISA or RT-PCR/PCR (for relevant grapevine harmful viruses and phytoplasmas).
12. Grafting on appropriate rootstock, if needed.

Tests for presence of selected pathogens by ELISA or PCR-based methods by official laboratories.

Irrespective of the country of origin of the plant material, the testing shall use appropriate laboratory methods and, where appropriate, indicator plants for the detection of at least the following harmful organisms:

- a) Blueberry leaf mottle virus.
- b) Grapevine Flavescence dorée MLO and other grapevine yellows.
- c) Peach rosette mosaic virus.
- d) Tobacco ringspot virus.
- e) Tomato ringspot virus (strain “yellow vein” and other strains).
- f) *Xylella fastidiosa* (Well & Raju).
- g) *Xylophilus ampelinus* (Panagopoulos) WILLEMS *et al.*
- h) Ajinashika disease (for ETC where the disease is known to be present).
- i) Grapevine stunt (for ETC where the disease is known to be present).
- j) Summer mottle (for ETC where the disease is known to be present).

Some other pathogens do not belong to the quarantine organisms but they can negatively influence the evaluation of phenotypic traits:

- a) Grapevine fanleaf virus (GFLV).
- b) Arabis mosaic virus (ArMV).
- c) Grapevine leafroll associated virus 1 (GLRaV1).
- d) Grapevine leafroll associated virus 3 (GRLaV3).
- e) Grapevine fleck virus (GFkV).